
PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Strategies To Limit Disease Effects On Estuarine Survival

BPA project number: 20052

Contract renewal date (mm/yyyy): _____ ☐ **Multiple actions?**

Business name of agency, institution or organization requesting funding
Oregon State University, National Marine Fisheries Service

Business acronym (if appropriate) OSU, NMFS

Proposal contact person or principal investigator:

Name	<u>Jerri L. Bartholomew</u>
Mailing Address	<u>Dept. of Microbiology, Nash Hall 220, OSU</u>
City, ST Zip	<u>Corvallis, OR 97331-3804</u>
Phone	<u>541-737-1856</u>
Fax	<u>541-737-0496</u>
Email address	<u>bartholj@bcc.orst.edu</u>

NPPC Program Measure Number(s) which this project addresses

7.2 Improve Existing Hatchery Production (7.2D.4 - Improve Fish Health), 7.4H
Reintroduction of Anadromous Fish in the Upper Cowlitz River Basin,

FWS/NMFS Biological Opinion Number(s) which this project addresses

Other planning document references

Consideration of Ocean Conditions in the Columbia River Basin Fish and Wildlife
Program

Short description

Determine the impact of pathogens on fish survival in the estuary and examine fish rearing,
release, and treatment strategies for decreasing pathogen effects.

Target species

Spring chinook salmon (*Oncorhynchus tshawytscha*)

Section 2. Sorting and evaluation

Subbasin

Estuary

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
<input checked="" type="checkbox"/> Anadromous fish <input type="checkbox"/> Resident fish <input type="checkbox"/> Wildlife	<input checked="" type="checkbox"/> Multi-year (milestone-based evaluation) <input type="checkbox"/> Watershed project evaluation	<input type="checkbox"/> Watershed councils/model watersheds <input type="checkbox"/> Information dissemination <input type="checkbox"/> Operation & maintenance <input type="checkbox"/> New construction <input checked="" type="checkbox"/> Research & monitoring <input type="checkbox"/> Implementation & management <input type="checkbox"/> Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description
_____	_____
_____	_____
_____	_____
_____	_____

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
9063	Ocean survival of salmonids relative to migrational timing, fish health..	Our proposed study in concert with this ongoing project will provide for a comprehensive evaluation of the influence of pathogens on salmonid health and survival as they migrate from their freshwater to ocean environments.
9102800	Monitoring smolt migration of wild Snake River spring/summer chinook salmo	By collecting fish in the estuary, this project will further the monitoring program by providing information on estuarine survival of chinook salmon.
9801001	Grande Ronde Basin spring chinook salmon captive broodstock program	Research on a vaccine for BKD and immunostimulant effects may provide

		therateutants necessary for maintaining health of captive broodstock.
9600600	PATH	Provides critical and empirical data for modeling survival in PATH

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Prevalence of pathogens in outmigrants prior to entering and as they leave the estuary	a	Fish sampling
		b	Determine pathogen prevalence
		c	Analysis of data
2	Specific pathogen studies to determine the effects of salt water on disease progress	a	Determine the effects of different stages of infection by EIBSV on salt water adaptation
		b	Determine the effects of different levels of C. shasta infection on survival of chinook salmon in salt water
		c	Determine the effect of BKD infection and stage of smoltification on salt water adaptation
3	Evaluation of a BKD vaccine and immunostimulants for decreasing pathogen effects in the estuary	a	Efficacy of a vaccine against Renibacterium salmoninarum
		b	Efficacy of B-glucans for enhancing the immune response against EIBSV and Ceratomyxa shasta

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	10/1999	9/2002	Prevalence of specific pathogens in chinook salmon entering and leaving the estuary.	___X__	45.00%
2	10/1999	5/2001	Data on pathogen impact in estuary relative to fresh water, development of management strategies	___X_	55.00%
3	10/2000	9/2002	Determine efficacy of BKD vaccine and immunostimulants	___X	0.00%
				Total	100.00%

Schedule constraints

Completion date

2002

Section 5. Budget

FY99 project budget (BPA obligated): \$0

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	Bartholomew \$12,172; Bootland \$12,540; Technicians (2) \$56,000 GRA \$12,996; hourly \$3,200	%29	96,908
Fringe benefits	@41% to 51%	%11	38,290
Supplies, materials, non-expendable property	PCR reagents, tissue culture, ELISA kits, bacteriological media, supplies.	%5	18,000
Operations & maintenance	tank charges	%1	4,776
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	_____	%0	0

NEPA costs	_____	%0	0
Construction-related support	_____	%0	0
PIT tags	# of tags: _____	%0	0
Travel	project-related travel and travel to 1 national meeting per year for each OSU PI	%1	2,500
Indirect costs	@43%	%24	79,754
Subcontractor	NMFS - Arkoosh and Jacobson (see itemized budget_	%26	87,054
Other	tuition	%2	6,896
TOTAL BPA FY2000 BUDGET REQUEST			\$334,178

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount (\$)
NMFS	salaries (1 month plus benefits for all project personnel)	%6	19,527
_____	_____	%0	0
_____	_____	%0	0
_____	_____	%0	0
Total project cost (including BPA portion)			\$353,705

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	\$343,863	\$355,445	\$0	\$0

Section 6. References

Watershed?	Reference
<input type="checkbox"/>	Arkoosh, M. R., E. Clemons, A. N, Kagley, R. Olson, P. Reno, E. Casillas and J. E. Stein. 1998. Effect of pollution on fish diseases: Potential impacts on salmonid populations. J. Aquat. Anim. Health. 10:182-190.
<input type="checkbox"/>	Banner, C.R., Long, J.J., Fryer, J.L, Rohovec, J.S. 1986. Occurrence of salmonid fish infected with Renibacterium salmoninarum in the Pacific Ocean. J. Fish Dis. 9: 273-275.
<input type="checkbox"/>	Bartholomew, J. L., J. L. Fryer and J. S. Rohovec. 1992. Impact of the myxosporean parasite, Ceratomyxa shasta, on survival of migrating Columbia River basin salmonids. In: Proc. 19th US. - Japan meeting on Aquaculture

	ISE, Japan pp 33-4 1.
<input type="checkbox"/>	Bootland, L.M., P. Dobos and R.M.W. Stevenson. 1995. Efficacy of immunization of adult brook trout in preventing the IPNV carrier state and vertical transmission. <i>J. Fish Dis.</i> 18: 449-458.
<input type="checkbox"/>	Brusland, L., V. Watral, J. Rohovec and P. Reno. 1994. Purification of the causative agent of erythrocytic inclusion body syndrome (EIBS) in salmonids. <i>Fish Health Section Newsletter</i> , 22:14-15.
<input type="checkbox"/>	Clarke, W. C., 1982, Evaluation of the seawater challenge test as an index of marine survival. <i>Aquaculture</i> , 28:177-183.
<input type="checkbox"/>	Daly, J.G., A.R. Moore and G. Olivier. 1995. A colorimetric assay for the quantification of brook trout (<i>Salvelinus fontinalis</i>) lymphocyte mitogenesis. <i>Fish Shellfish Immunol.</i> 5: 265-273.
<input type="checkbox"/>	Elliott, D. G., R. J. Pascho, L. M. Jackson, G. M. Matthews, J. R. Harmon. 1997. <i>Renibacterium salmoninarum</i> in spring-summer chinook salmon smolts at dams on the Columbia and Snake Rivers. <i>J. Aquat. Anim. Health.</i> 9:114-126.
<input type="checkbox"/>	Evelyn, T.P.T., J. E. Ketcheson, L. Prosperi-Porta. 1988. Trials with antibacterial kidney disease vaccines in two species of Pacific salmon. In: <i>Conf. Handbook. Internat. Fish Health. Conf., Vancouver, B.C., Fish Health Section, Am. Fish. Soc., WA</i>
<input type="checkbox"/>	Fryer, J.L. and J. E. Sanders. 1981. Bacterial kidney disease of salmonid fish. <i>Ann. Rev. Microbiol.</i> 35:273-298.
<input type="checkbox"/>	Gulland, F.M.D. The impact of infectious diseases on wild animal populations – A review. In <i>Ecology of Infectious Diseases in Natural Populations</i> . Grenfell, B.T. and A.P.Dobson (eds), Cambridge University Press, New York, p 20-51.
<input type="checkbox"/>	Haner, P.V., J.C. Faler, R.M. Schrock, D.W. Rondorf and A.G. Maule. 1995. Skin reflectance as a nonlethal measure of smoltification for juvenile salmonids. <i>N. Amer. J. Fish. Manage.</i> 15:814-822.
<input type="checkbox"/>	Hardie, L.J., T.C. Fletcher and C.J. Secombes. 1994. Effect of temperature on macrophage activation and the production of macrophage activating factor by rainbow trout (<i>Oncorhynchus mykiss</i>) leucocytes. <i>Dev. Comp. Immunol.</i> 18: 57-66.
<input type="checkbox"/>	Kaattari, S.L., P. Turaga, G.D. Weins. 1989. Development of a vaccine for bacterial kidney disease in salmon. Final Report, August 1989. Bonneville Power Administration, Portland, OR. 310 p.
<input type="checkbox"/>	Magnusson, H.B., O.H. Fridjonsson, O.S. Andresson, E. Benediktsdottir, S. Gudmundsdottir and Anderson. 1994. Rs, the causative agent of bacterial kidney disease in salmonid fish, detection by reverse transcription-PCR. <i>J. Appl. Environ. Microbiol.</i> 60: 4580-4
<input type="checkbox"/>	Moles, A. 1997. Effect of bacterial kidney disease on saltwater adaptation of coho salmon smolts. <i>J. Aquat. Anim. Health.</i> 9:230-233.
<input type="checkbox"/>	Munro, A.L.S., D.W. Bruno. 1988. Vaccination against bacterial kidney disease. In: Ellis, A.E. (ed.) <i>Fish Vaccination</i> . Academic Press, San Diego,

	CA. pp. 124-134.
<input type="checkbox"/>	NRC (National Research Council) 1996. Upstream: Salmon and Society in the Pacific Northwest. Committee on Protection and Management of Northwest Salmonids, Bd. Env. Studies and Tox., Comm. Life Sci., 452 pp. Natn'l Acad. Press. Washington D. C.
<input type="checkbox"/>	NWPPC. 1997. Consideration of Ocean Conditions in the Columbia River Basin Fish and Wildlife Program. May 29, 1997. (Internet access).
<input type="checkbox"/>	Palenzuela, O., G. Trobridge and J. L. Bartholomew. Development of a polymerase chain reaction diagnostic assay for <i>Ceratomyxa shasta</i> , a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms. In Press.
<input type="checkbox"/>	Pascho, R. J., D. G. Elliot and S. Achord. 1993. Monitoring of the in-river migration of smolts from two groups of spring chinook salmon with different profiles of <i>Renibacterium salmoninarum</i> infection. Aquacult. Fish Manage. 24:163-169.
<input type="checkbox"/>	Pearcy, W. G. 1992. Ocean ecology of north Pacific salmonids. University of Washington Press, Seattle, 179 pp.
<input type="checkbox"/>	Piacentini, S. C., J. S. Rohovec and J. L. Fryer. 1989. Epizootiology of erythrocytic inclusion body syndrome. J. Aquat. Anim. Health. 1:173-179.
<input type="checkbox"/>	Piganelli, J.D. 1994. Ph.D. Thesis: Development of enteric protected vaccines for aquaculture. Oregon State University, Corvallis, OR.
<input type="checkbox"/>	PNFHPC. 1994. PNFHPC survey to rank the importance of selected fish diseases and research approaches that might be taken to reduce their impact. Final report. Feb. 1994.
<input type="checkbox"/>	Ratliff, D. E. 1981. <i>Ceratomyxa shasta</i> : Epizootiology in chinook salmon of central Oregon. Trans. Amer. Fish. Soc. 110:507-513.
<input type="checkbox"/>	J. E. Sanders, J. J. Long, C. K. Arakawa, J. L. Bartholomew and J. S. Rohovec. 1992. Prevalence of <i>Renibacterium salmoninarum</i> among downstream-migrating Columbia River salmonids. J. Aquat. Anim. Health. 4:72-75.

PART II - NARRATIVE

Section 7. Abstract

Among the critical uncertainties in the life cycle of hatchery salmon are the factors affecting their survival in the estuary. The numerous pathogens and diseases affecting salmonids in hatcheries and during the freshwater life cycle phase are well understood. However, there is little known about the impact of pathogens on the health and survival of fish during the transition to the saltwater phase of their life cycle. Pathogens of special concern in the Columbia River Basin (CRB) are the virus which causes erythrocytic inclusion body syndrome (EIBS); *Ceratomyxa shasta*, a parasite enzootic throughout much of the CRB, and the ubiquitous *Renibacterium salmoninarum*, the agent causing bacterial kidney disease (BKD). We hypothesize that the final

stress of migrating into the estuary may result in fish mortality even if pathogen levels were low at release. There are multiple strategies which could be implemented in hatcheries to decrease the effects of pathogens. **We propose to assess whether the above three pathogens impact fish populations by 1) monitoring pathogen prevalence in smolts migrating through the Columbia River estuary and 2) examining in laboratory experiments if pathogens alter fish survival during adaptation to salt water. We also propose to test methods (e.g. immunostimulants and vaccines) which will potentially help hatchery managers to increase survival of fish after release.**

Section 8. Project description

a. Technical and/or scientific background

A major goal of the Northwest Power Planning Council's (NWPPC) Fish and Wildlife Program (Program) is to double salmon and steelhead runs in the Columbia River Basin (CRB). Achievement of this goal will require multiple approaches, including hatchery propagation of native fish and development of captive broodstock programs. Disease has been acknowledged as a limiting factor in hatchery production and numerous pathogens have been detected in migrating hatchery and wild salmon. Efforts to rebuild anadromous salmon runs will require a comprehensive program that focuses on all life history stages and identifies all opportunities to increase survival. In such an approach, a focus on the poorly understood transition of hatchery smolts to sea water and the influence of disease on this process is clearly needed.

Our knowledge of the effects of pathogens on fish entering sea water has been extrapolated from only a few studies. Research conducted in the CRB by this laboratory demonstrated that more than 20% of 2,800 chinook and coho salmon and steelhead trout smolts collected prior to entering the estuary during 1983-84 were infected with *R. salmoninarum* (Rs), the cause of BKD (Sanders et al. 1992). In this same study, more than 10% of the captured chinook salmon held in sea water died from ceratomyxosis (Bartholomew et al. 1992). A study evaluating seawater challenges as an index of marine survival (Clarke 1982) demonstrated that infestation with a cestode parasite affected the osmoregulatory capabilities of the infected fish. The period of saltwater transition represents a critical uncertainty in life cycle models and the above studies demonstrate that pathogens are likely to have a significant effect on the survival of Columbia River salmon during this life phase.

The National Research Council's review of the Pacific Northwest salmon problem states that "disease is thought to be directly and indirectly responsible for substantial post-release mortality of hatchery fish" (NRC, 1996). Of the many salmonid pathogens, three that are of major concern for outmigrating salmonids are the bacterium that causes bacterial kidney disease (BKD), the virus that causes erythrocytic inclusion body syndrome (EIBS), and the parasite *Ceratomyxa shasta*. Fish disease research needs were specifically addressed in the final report of the Research Priority Subcommittee of the Pacific Northwest Fish

Health Protection Committee. Priorities for research on BKD included new drug and chemical research and a better understanding of the epizootiology of the infection in salt water. High priorities for EIBS research included understanding the epizootiology of the disease, development of diagnostic reagents and integrated fish health management studies. The report acknowledged that *Ceratomyxa* is an important factor in the survival of migrating smolts in the lower Columbia River, and that research should focus on epizootiology and integrated fish health management. **We will address these research needs in the proposed study by: 1) determining the prevalence of EIBSV, Rs and *C. shasta* in spring chinook salmon collected prior to entering and during their migration through the estuary, 2) assessing the effect of these pathogens on the survival of chinook salmon acclimating to estuarine conditions and 3) determining if vaccines and immunostimulants, or changes in management strategies could be used to mitigate pathogen effects.**

Classically, to determine the effects of these pathogens on salmonid populations, one would quantify host reproduction and survival (Gulland, 1995). However, these parameters are extremely difficult to assess once the salmon have left a hatchery and entered a dynamic ecosystem. In this environment, the quantification of dead salmon by the recovery of fish carcasses is unfeasible. These constraints may contribute to underestimating the mortality from disease and in attributing disease-related mortality to secondary factors like predation. **Therefore, we propose to survey for these three pathogens in juvenile salmon temporally and spatially as they outmigrate from fresh water to the estuarine environment. This will allow us to determine if these pathogens have the potential to influence the salmon's ability to successfully survive salt water entry.**

The agent that causes EIBS is a virus that replicates in the cytoplasm of red blood cells and causes anemia in the infected fish. The syndrome has been observed in chinook and coho salmon smolts at hatcheries in the CRB. Advances in knowledge of EIBSV have been slow, primarily because of the inability to culture this organism in an established cell line and the lack of suitable diagnostic reagents. Studies on EIBS in the CRB by Piacentini et al. (1989) demonstrated that the disease progresses through five stages. In the initial incubation stage, no pathology is evident but viral inclusions can be detected. In subsequent stages of the disease, the virus replicates, causing lysis of the red blood cells and anemia. If the fish survives and the infection is not complicated with other pathogens, a recovery phase begins. The progression through these phases is accelerated with increased temperatures. **The proposed study will provide information on the ability of the fish to acclimate to sea water successfully during the different stages of the infection, allowing hatchery managers to make informed decisions on when to release infected fish.**

Renibacterium salmoninarum (Rs) is widely recognized as a primary deterrent to the successful culture of salmon in the Pacific Northwest. A great deal of research into hatchery practices such as erythromycin therapy, brood stock segregation or culling, and optimal rearing density have decreased the prevalence of BKD in many hatcheries.

However, these reductions at the hatcheries have not necessarily resulted in lower prevalence or levels of the bacterium in these same groups after release (Elliott et al. 1997). Unable to eradicate this pathogen, hatchery managers must balance the benefits of early release with the loss from disease if the stress of infection affects osmocompetence and therefore, survival. A study conducted in this laboratory (Sanders et al. 1992) demonstrated that subyearling chinook salmon seined from the river prior to entering the estuary and transferred to salt water had a much higher prevalence of Rs (46%) than their cohorts held in fresh water (9%). A recent study by Moles (1997) demonstrated that infected fish transferred to salt water after extended exercise suffered osmoregulatory impairment and were unable to adapt. These and other studies (Banner et al. 1986; Fryer and Sanders 1981) indicate that Rs may be a more efficient pathogen in salt water. **In the proposed research, the relationship between smoltification and BKD will be investigated.**

The myxosporean, *Ceratomyxa shasta*, is enzootic in the CRB and has been recognized as a serious contributor to mortality of juvenile and adult salmonids within the region (Sanders et al. 1997; Bartholomew et al. 1992). Most strains of salmonids in the CRB have developed some resistance to this parasite and this differential resistance has been used extensively in management. However, resistance can be overwhelmed and studies in the Deschutes and Willamette Rivers demonstrate that *C. shasta* has a substantial impact on salmonid populations (Ratliff 1981; Bartholomew et al. 1992). When causes of mortality among winter steelhead trout in the Willamette River were examined, between 30% and 92% of the outmigrants succumbed to ceratomyxosis during each of the four study years (R.A. Holt, Senior Pathologist, Oregon Department of Fish and Wildlife, personal communication). Efforts to determine the effects of entering salt water on the infectious process have yielded conflicting results, possibly because the extent of the infection at transfer was not known. However, some of the studies indicated that mortality decreased in certain groups. If so, this may indicate that fish at certain stages of infection are able to suppress the parasite and survive in saltwater. Survival with infection by *C. shasta* is likely influenced by the duration of exposure to the parasite, the parasite concentration and river water temperatures. **The proposed research will determine if the stage or level of infection by *C. shasta* affects survival in salt water.**

Alternative strategies for disease control include administering therapeutants or vaccines that would protect the fish after release. Erythromycin has been effectively used for treatment of BKD; however, it is toxic with prolonged use and its efficacy in improving saltwater survival has not been tested. Vaccination is a desirable alternative to antibiotics for controlling BKD. However, many vaccines trials have shown only marginal success (Evelyn et al. 1988; Munro & Bruno 1988). These vaccines may have failed because they included the immunosuppressive Rs major p57 surface protein (Kaattari et al. 1989). Recent research by Piganelli (1994) demonstrated that an oral BKD vaccine lacking this protein is efficacious in coho salmon and Dr. Bootland is currently investigating the efficacy of this oral vaccine in chinook salmon. An alternative to specific vaccines and antibiotic treatments is the administration of immunostimulants that enhance the non-specific defenses of fish. Considerable research on the immunostimulant β -glucan has

demonstrated increased resistance to infection by several bacterial pathogens after addition of the compound to the diet. The advantages of this treatment, if proven effective, are that immunostimulants have the potential of enhancing resistance to a variety of pathogens, are relatively inexpensive to orally administer, non-toxic and will not contribute to the spread of antibiotic resistance genes. **In the proposed research, the efficacy of a BKD vaccine will be measured for specifically increasing survival in fish infected with Rs, and the administration of an immunostimulant will be investigated as a strategy for non-specifically enhancing resistance to EIBS and *C. shasta*.**

Hatchery management practices have been effective in decreasing the effects of pathogens on salmonid survival. These practices include decreasing fish densities, broodstock segregation or culling for vertically transmitted pathogens like Rs, and release of healthy fish which will endure the stresses of migration. Survival could be further enhanced by timing release of the fish to coincide with periods of lowered water temperatures or increased flows, or by monitoring infection status and releasing at an infection level where the stress of migration is likely to have the least effect. However, taking advantage of additional management options will require a better understanding of how transition to salt water affects the disease process.

b. Rationale and significance to Regional Programs

Successful conservation of salmonid populations requires an understanding of the factors that can alter survival and reproduction. Salmonid year-class strength is believed to be predominantly determined early in their life history (Pearcy, 1992). However, the contribution and interaction of ecological factors influencing survival of salmon are poorly understood. Changes in habitat resulting from human influences and natural environmental variables can influence ecological interactions and alter salmonid estuarine and early ocean survival. Bacterial, viral and parasitic diseases are endemic problems for juvenile salmon in the Pacific Northwest, including the CRB. Diseases are one of the ecological factors, enhanced by poor habitat quality, which can modulate salmonid distribution and abundance. This project will provide information necessary for assessing the contribution of disease to survival in the estuarine and ocean phases of the salmonid life cycle.

This proposal will also produce data significant to regional programs. For example, reintroduction and enhancement programs in the Cowlitz, Deschutes and Willamette Rivers may be severely affected by the presence of *C. shasta*. This project will provide information necessary to insure sound application of biological principles during reintroduction. Development of a BKD vaccine and methods for treating with immunostimulants is relevant to all regional supplementation programs.

c. Relationships to other projects

This project would complement two ongoing studies currently funded by BPA and the National Marine Fisheries Service (NMFS). One study entitled "Relationships between

pathogens, natural and anthropogenic factors that influence disease prevalence, and survival of juvenile salmon in the estuarine and nearshore ocean environment” is funded by the Recovered Protected Species Office (RPSO) of the NMFS. This work examines pathogen prevalence in juvenile coho and fall chinook salmon and the influence of natural and anthropogenic factors on estuarine and riverine habitat properties (Arkoosh et al. 1998). This proposed BPA project would supplement the preliminary pathogen survey data collected from juvenile chinook salmon from the CR. As collaborators in the proposed BPA project, NMFS would amend their formal application for a Permit for Scientific Purposes under the Endangered Species Act of 1973 to take into account endangered or threatened species collected for this proposed BPA study.

Another study (BPA project 9063) entitled “Ocean survival of juvenile salmonids in the Columbia River Plume” is examining the effects of health parameters (growth, pathogen prevalence and bioenergetics) on coho and chinook salmon survival in relation to nearshore oceanographic features associated with the CR plume. Our proposed BPA study in concert with the ongoing project described above will provide for a comprehensive evaluation of the influence of pathogens on salmonid health and survival as they migrate from fresh water to their ocean environments.

d. Project history (for ongoing projects)

This is a new project.

e. Proposal objectives

- 1. Assess the prevalence and levels of Rs, *C. shasta* and EIBSV in outmigrating salmonids collected prior to entering the estuary and as they migrate through the estuary.** A decrease in the prevalence of these pathogens indicates either that pathogen levels, and therefore losses to disease, are decreased during the estuarine life phase, or alternatively, that fish die in or as they enter the estuary. Data obtained from this objective combined with results of laboratory exposures, will enable us to assess the impact of these pathogens on salmonids migrating through the estuary.
- 2. Specific pathogen studies: Determine the effects of transfer to salt water on the progress of EIBS, Ceratomyxosis, and BKD.** The three pathogens selected for this portion of the study have been documented as causing disease in chinook salmon within the CRB. The effects of these pathogens on fish during their freshwater phase is well documented; however, studies of their effects on salmon in salt water have not provided clear evidence of their impact. Determining the effects of each pathogen at release under different conditions will provide data necessary for assessing pathogen effects in the estuary.
- 3. Efficacy of a BKD vaccine and immunostimulant for decreasing pathogen effects.** Another method for decreasing disease losses is to enhance host responses to the pathogens. This may be done either specifically, using a vaccine, or non-specifically, with general immunostimulants. In this objective we will test the efficacy

of a vaccine against BKD and evaluate the immunostimulant β -glucan for decreasing the effects of EIBS and *C. shasta*.

f. Methods

Objective 1. Assess the prevalence of Rs, *C. shasta* and EIBSV in outmigrating salmonids collected prior to entering the estuary and as they migrate through the estuary into salt water.

Task 1a. Fish sampling.

Target juvenile spring chinook salmon will be collected by seining during daylight hours. Due to patterns of salmon movement, sampling will generally be done on outgoing tides using a beach seine, mid-water purse seine, or fyke net. A sample size of 60-80 fish will be collected biweekly through April and May from a freshwater site (Jones Beach) just prior to the estuary and from a saltwater site (Clatsop Spit) at the mouth of the CR estuary. Each site will be sampled four times during outmigration; up to 600 fish will be sampled each year.

Task 1b. Determination of pathogen prevalence.

After the salmon are euthanized with MS-222, samples will be collected for the analysis of pathogen prevalence and intensity. Each fish will receive a numbered tag for identification and all samples taken from that fish identified by that number. A blood sample will be taken immediately and a smear made for identification of the EIBSV. Measurements of length, weight and general condition will be made before necropsy and any identifying tags or clips recorded. For determination of infection by *C. shasta*, a portion of the posterior intestine will be placed in 1 ml of lysis buffer for later analysis using the polymerase chain reaction (PCR). A sample of kidney will be excised using flamed forceps and a scalpel and placed in a second vial of lysis buffer for determination of Rs infection by PCR. In addition to examination for these specific pathogens, an agar plate will be inoculated with kidney tissue to determine the presence of other bacterial pathogens.

Samples will be returned to the laboratory on ice. Blood smears will be stained as described by Piacentini et al. (1989) and examined for viral inclusions. Intestinal samples will be processed for the *C. shasta*-specific PCR as described by Palenzuela et al. (in press). Kidney samples will be processed for the Rs-specific PCR as described by Magnusson et al. (1994).

Task 1c. Analysis of data

Data on pathogen presence will be compiled in a database for comparison of sample sets. Samples will be analyzed for multiple infections as well as for differences in prevalence between sites and dates. The data will be examined statistically by analysis of variance (ANOVA) and t-tests at a significance level of 5%. These data will also be added to a larger database managed by the NMFS through support from the RPSO.

Objective 2. Specific pathogen studies: Determine the effects of transfer to salt water on the progress of EIBS, Ceratomyxosis, and BKD.

Task 2a. Determine the effects of different stages of EIBS on the survival of fish as they enter the estuary.

Fish: Spring chinook salmon from a CRB hatchery will be used in all laboratory experiments. The fish will be maintained in specific fish-pathogen-free water at a temperature of 12C.

Virus collection: Because the virus causing EIBS cannot be cultured, material for infection will be obtained directly from kidney, spleen and blood collected from naturally infected fish. The tissues will be homogenized and the virus partially purified (Bruslind et al. 1994).

Challenge experiments: Fish will be anesthetized with MS222 and artificially infected by an intraperitoneal injection with partially purified virus (Piacentini et al. 1989). Fish will be divided into 16 groups of 60 fish. At 10 days, two groups of 60 fish will be anesthetized in MS-222 and given an intraperitoneal injection with semi-purified virus and two groups of 60 fish will be similarly injected with PBS as negative controls. At 20, 30 and 40 days, two groups will be injected with virus and two groups will be injected with buffered saline (PBS). At 45 days, 8 groups consisting of one virus-injected and PBS group injected at each time interval will be transported to Hatfield Marine Science Center (HMSC) and acclimated over a 5-day period to salt water. To mimic the stress of transport, fish in the 8 groups remaining at the SDL will be netted, placed in transport containers supplied with oxygen, and held for the same amount of time as required to transport fish to HMSC. At each facility, fish will be held in identical volumes of water.

Mortality and infection will be monitored for 30d post-transfer and the prevalence and level of infection will be assessed by examination of blood smears from all fish.

Data Analysis: Data from each group of fish will be presented as the cumulative percent mortality, the mean day to death (MDD), the prevalence of infection and pathogen load. Data will be statistically analyzed by ANOVA and t-tests at a significant level of 5%. Comparison of trends in mortality and MDD between the different pathogens will be conducted by simple correlation analysis.

Task 2b. Determine the effect of the stage of ceratomyxosis and different levels of infection on survival of chinook salmon as they enter the estuary.

Fish: Two strains of chinook salmon will be used for these challenges: a *C. shasta*-susceptible strain (from a coastal hatchery) and a *C. shasta*-resistant strain (from a Willamette River hatchery). All fish will be reared at the SDL until exposure.

Challenge: A group of 360 fish of each strain will be naturally exposed to *C. shasta* for 5 days by holding in live cages in the Willamette River, where the parasite is enzootic. A group of 120 fish per strain will not be exposed. The effects of entering salt water with an early versus a late stage of infection will be determined by transporting 60 exposed fish of each strain to the HMSC at intervals of 5, 10 and 20 days post exposure and acclimating them to salt water over 5 d. An additional 60 exposed fish for each interval will be held at the SDL, again, after reproducing the stress of transport. A group of 60 unexposed fish will be held at the SDL and the HMSC as negative controls.

The effects of the level of *C. shasta* infection on estuarine survival will be determined by exposing 240 fish from each strain for 3 d and for 10 d. Upon removal from the river, half of each strain will be transported to the HMSC and acclimated to salt water. A control group of 60 uninfected fish per strain will be held in fresh and an equal number of uninfected fish acclimated to salt water.

For both experiments, mortality and infection will be monitored for 80 d post-transfer and the *C. shasta* incidence in all fish assessed by PCR.

Data Analysis: Data will be analyzed as described under Task 2a.

Task 2c. Determine the effect of BKD infection and stage of smoltification on the survival of chinook salmon entering the estuary.

Fish: Spring chinook salmon pre-smolts will be obtained from ODFW hatcheries in which broodstock culling programs are established and where levels of infection are low (e.g. Willamette Hatchery). The fish will be held at the SDL for at least 1 month prior to the experiment and 60 fish examined by ELISA (DiagXotics) to determine existing levels of infection.

Challenge: Pre-smolts (360 fish) will be injected intraperitoneally with a standard dose of Rs, predetermined in preliminary experiments. An equal number of fish will be injected with the same volume of PBS as a control. Degree of smoltification will be assessed using a gill ATPase assay (Haner et al. 1995). A standard curve of predicted values will be obtained during the first study year and will be used to determine early and late stages of smoltification. Groups of 60 infected and control fish will be transferred to the HMSC prior to smoltification. Acclimation to salt water will be over a 5-day period as described. At the beginning of smoltification, the second group of 60 infected and control fish will be transported and adapted to salt water. This will be repeated for a final group in the late stages of smoltification. At each transport time, groups of 60 infected and control fish maintained at the SDL will be subjected to transport stress as described. Mortality will be monitored in all groups for at least 90 days post transfer and BKD levels in all fish will be assessed by ELISA.

Data Analysis: Data will be analyzed as described under Task 2a.

Objective 3. Evaluation of a BKD vaccine and immunostimulants for decreasing pathogen effects in the estuary.

Task 3a. Efficacy of a vaccine against *Renibacterium salmoninarum* for decreasing the loss to BKD in fish entering salt water.

Administration of vaccine and challenge with Rs: The oral BKD vaccine will be fed to 400 juvenile spring chinook salmon at a rate of 100 µg/fish/day for 30 days (Piganelli 1994). Another 400 control fish will be fed the same diet without vaccine. Half of the vaccinated and control fish will be challenged at 45 days by a 24-h immersion in a predetermined dose of bacteria. Half of the fish will remain unchallenged. This will result in 4 groups of 200 fish each. At 3 weeks post-challenge, half of each of the 4 groups will be transferred to the HMSC and acclimated to salt water as described. Fish mortality in all groups will be monitored for 4 months post-transfer. A group of 40 additional fish will be immunized and an equal number of fish will act as negative

controls for use in measuring immune responses. At 5 days prior to challenge, the immune responses of twenty fish in each of the two groups will be measured as outlined below, and the remaining 20 fish per group will be sampled at 21 days after transfer to salt water.

Analysis of data: Mortality data will be collected and analyzed as described under Task 2a.

Immunoassay Methods: Fish will be bled, the anterior kidney removed and antibody titers will be measured by ELISA. Leukocytes and macrophages will be purified from blood and anterior kidney (Bootland et al. 1995), resuspended in L15 medium, counted and diluted for the following assays.

For measuring lymphocyte proliferation, purified blood leukocytes will be stimulated with the T cell mitogen ConA or specific killed pathogens. Lymphocyte proliferation will be measured using the MTT colorimetric assay described by Daly et al. (1995). The stimulation index (S.I.) will be calculated using the following equation:

$$\text{S.I.} = \text{OD cells incubated with mitogen or antigen} / \text{OD cells in medium.}$$

To monitor macrophage activation with and without the addition of macrophage activating factor, intracellular superoxide anion production will be measured using the nitroblue tetrazolium (NBT) assay (Hardie et al. 1994). The results will be expressed as an activation index:

$$\text{Activation Index} = \text{OD}_{650} \text{ cells} + \text{stimulator} / \text{OD}_{650} \text{ control cells.}$$

Significant differences in macrophage activation and lymphocyte stimulation between groups will be identified by ANOVA and Fisher's LSD test at a significance level of 5%.

Task 3b. Efficacy of β -Glucans for enhancing the immune response against EIBSV and *Ceratomyxa shasta* and increasing survival in salt water.

Administration of glucans: β -glucan incorporated in the diet will be fed to 840 spring chinook salmon at a rate of 2% body wt/fish/day for 30 days and 840 fish will be fed normal diets as untreated controls.

Challenge with *C. shasta*: For the *C. shasta* challenge, 200 fish fed β -glucan and 200 untreated fish will be exposed to the parasite for 5 d, as described. Another 200 fish fed β -glucan and 200 untreated fish will serve as unexposed controls. This will result in 4 groups of 200 fish each: treated and exposed, untreated and exposed, treated and unexposed, untreated and unexposed. Following exposure, 100 fish in each group will be transferred to the HMSC and acclimated to salt water, and the other 100 fish per group will remain in fresh water at the SDL. Mortalities will be monitored in all groups for 90 days post-transfer.

Challenge with the EIBSV: For the EIBSV challenge, 200 β -glucan-treated fish and 200 untreated fish will be injected with a dose of the virus as determined in Obj 2. The remaining 200 treated and 200 untreated fish will serve as uninjected controls. Data from Obj 2 will be used to determine the time post-injection when fish will be transferred to salt water. For example, if it was determined that 30-days post-infection is the most critical time for the fish, that period will be used in this study. Half of each of the 4 groups will be transferred to the HMSC and acclimated to salt water. The

remaining fish will be held in fresh water at the SDL. Mortalities will be monitored for 30 days post-transfer.

Immune Function Assays. As described in Task 3a, assays of immune function will be used to assess if the treatment actually boosted the host response to EIBSV and *C. shasta*. The numbers of fish, experimental design, and assays used will be as described above. However, because infections by these pathogens are of a more acute nature, assays will be performed at 10 d after transfer to salt water rather than at 21 d.

g. Facilities and equipment

All *in vivo* experiments proposed in this study will be carried out at the Oregon State University Salmon Disease Laboratory (SDL) or the Hatfield Marine Science Center (HMSC). The SDL is a 9,000 ft² facility which is divided into three main sections: 1) the wet laboratory with an inside area for work with infectious agents and an outside area for uninfected animals, 2) the preparatory laboratory and 3) the dry laboratory. The SDL is supplied with specific pathogen-free water at an ambient temperature of 12°C. Effluent from the wet laboratory is treated with chlorine to ensure that no infectious agents exit via the water. The wet laboratory is separated into an outside holding area, where stock fish are held in 3-12 ft circular tanks, and the inside experimental area. Inside there are 144, 25-L tanks designed for holding smaller fish and 128, 100-L tanks. There is the capacity to both heat and chill water in this facility. The HMSC is a sister facility, with capabilities of supplying estuarine water. The Fish Disease Laboratory receives 220 gpm of specific pathogen free water that is pumped from Yaquina Bay, sand filtered and UV treated prior to delivery to the tanks. There are 48-2 ft and 30-4 ft circular tanks in the Fish Disease Laboratory. There is the capacity to chill the water. Before the effluent is released back into the Bay it is treated with charcoal and chlorine for removal of contaminants and virulent pathogens. The Fish Disease Laboratory has a capacity for fresh dechlorinated water (approximately 10 gpm). The dry laboratories contain facilities for sterile tissue culture work, bacterial culture, immunoassay and molecular diagnostics. Equipment such as a microfuge, a cell harvester, fume hoods, autoclaves, microscopes, refrigerators, and freezers are also available.

Laboratories of the principal investigators, in the Department of Microbiology, are equipped for research on infectious diseases of fish. Facilities include a biohazard room, a darkroom for photography, clean rooms for use in tissue culture and hybridoma work and a room for processing histological samples. The laboratories are well equipped with light microscopes, microscopes for fluorescence microscopy, inverted microscopes for tissue culture, thermocyclers for PCR, ELISA plate washer and reader, spectrophotometers, electrophoresis and western blotting equipment, preparatory and ultracentrifuges, scintillation counters and walk-in incubation chambers.

h. Budget

Personnel – Salaries (\$96,908) are requested. The 2 OSU co-PIs (0.33 FTE each) will manage all laboratory aspects, including design of experiments, logistics, writing reports and disseminating information. Two full-time technicians, one graduate student and an undergraduate will conduct challenge experiments, isolate and culture pathogens, perform immunological assays, and transport and care for fish.

Supplies, materials and non-expendable property – These costs (\$18,000 in FY00) will cover reagents and supplies for PCR assay (\$5.50/fish), bacteriological media, ELISA kits, plastics, immunological reagents and the costs for preparing the vaccine and treatment diets.

Operations and maintenance – Costs for holding fish for the first year will be \$4,776, based on tank charges at the SDL.

Travel – Monies are requested for project-related travel (\$1,500) to and from the HMSC and for project meetings, and for partial travel to 1 national meeting of the American Fisheries Society for each PI (\$1,000).

Indirect costs are calculated at the University rate of 43%.

Subcontractor – Monies to the NMFS (\$87,054) for 2 months of salary and benefits (\$39,054) plus the 8 collection trips and cost of holding fish (\$48,000). The 2 NMFS PIs will be responsible for all field aspects of the project.

Other – costs (\$6,896) are requested for student tuition.

Section 9. Key personnel

The principal investigator on this project is Dr. Jerri Bartholomew, assistant professor (0.33 FTE). Co-principal investigators are Dr. Linda Bootland (assistant professor, 0.33 FTE) and Dr. Jo-Ann Leong (Distinguished Professor and chair, Department of Microbiology; 0.05 FTE). Collaborators with the NMFS are Dr. Mary Arkoosh (NWFSC, NMFS, Environmental Conservation Division) and Dr. Kym Jacobson (NWFSC, NMFS, Fish Ecology Division). Drs. Bartholomew and Bootland will be responsible for the management of the laboratory portion of the project, including experimental design, conducting experiments, and data analysis. Dr. Bartholomew's research program focuses on parasitic infections of salmonid fish, especially on the myxosporeans which cause ceratomyxosis and whirling disease. Dr. Bootland's research is focused on the elucidation of virus-host interactions and vaccine development for fish pathogens. Dr. Leong's research program concentrates on the molecular biology and pathogenesis of salmonid viruses. Drs. Arkoosh and Jacobson will be responsible for the management of the field section of the project, including designing the survey, collection of fish from the river and the estuary, data analysis and report writing. Drs. Arkoosh and Jacobson are involved with research on natural and anthropogenic factors that influence disease prevalence, and survival of juvenile salmon in the estuarine and ocean environment. All these research programs involve the monitoring of infection and disease in salmonid fish.

CURRICULUM VITAE: Jerri L. Bartholomew

Assistant Professor, Senior Research
Department of Microbiology
Oregon State University, Nash Hall 220
Corvallis, OR 97331-3804

Work phone: 541-737-1856
Home phone: 541-758-7508

Education:

Ph.D. in Microbiology - Oregon State University, 1989
M.S. in Fisheries and Wildlife - Oregon State University, 1985
B.S. in Biology, minor in Marine Sciences - Pennsylvania State University, 1980

Professional Experience

1995-present	Assistant Professor, Senior Research, Dept. Microbiology, OSU
1994-1995	Instructor, Dept. Microbiology, OSU
1992-1993	Microbiologist, USFWS, NFRS, Seattle, WA
1990-1991	Research Associate, Department of Microbiology, OSU
1988-1990	Fishery Biologist, USFWS, NFRS, Seattle, WA
1982-1988	Graduate Research Assistant, Dept. of Microbiology, OSU

Present Funded Research

	Sponsor
Comparison of Three Isolates of <i>Piscirickettsia salmonis</i> with Differential Virulence for Salmonid Fish	Agricultural Research Foundation
Identification of Genetically Based Protective Responses against Infection by <i>Ceratomyxa shasta</i>	Sea Grant
Interactions Between <i>Ceratomyxa shasta</i> and its salmonid and Polychaete Host	USDA/Animal Health and Disease
Fish Disease Risk Assessment Study: Whirling Disease and Ceratomyxosis	Portland General Electric Company
Distribution and Seasonal Occurrence of <i>Myxobolus cerebralis</i> in the Lostine River, Oregon	Whirling Disease Initiative

Selected Publications

- Palenzuela, O., G. Trobridge and J. L. Bartholomew. Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. Diseases of Aquatic Organisms. In Press.
- Bartholomew, J. L. 1998. Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: a review. Journal of Aquatic Animal Health. 10:112-120.
- Moffitt, C. M., B. C. Stewart, S. E. LaPatra, R. D. Brunson, J. L. Bartholomew, J. E. Peterson, and K. H. Amon. 1998. Pathogens and diseases of fish in aquatic ecosystems: Implications in fisheries management. Journal of Aquatic Animal Health. 10: 95-100.
- Bartholomew, J. L., M. J. Whipple, D. G. Stevens and J. L. Fryer. 1997. Role of the freshwater polychaete, *Mayanukia speciosa*, in the life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmon and trout. American Journal of Parasitology. 83:859-868.
- J. E. Sanders, J. J. Long, C. K. Arakawa, J. L. Bartholomew and J. S. Rohovec. 1992. Prevalence of *Renibacterium salmoninarum* among downstream-migrating Columbia River salmonids. Journal of Aquatic Animal Health. 4:72-75.
- Bartholomew, J. L., J. L. Fryer and J. S. Rohovec. 1992. Impact of the myxosporean parasite, *Ceratomyxa shasta*, on survival of migrating Columbia River basin salmonids. Pages 33-41. in Proceedings of the 19th US. and Japan meeting on Aquaculture ISE, Mie Prefecture, Japan. October 29-30, 1990. US Dept. of Commerce NOAA Technical Report NMFS 111.
- Bartholomew, J. L., M. R. Arkoosh and J. S. Rohovec. 1991. Demonstration of the salmonid humoral response to *Renibacterium salmoninarum* using a monoclonal antibody against salmonid immunoglobulin. Journal of Aquatic Animal Health 3:254-259.

Curriculum vitae: LINDA M. BOOTLAND, Ph.D.

Education: B.Sc. Marine Biology 1982. Minors: Fisheries Science and Microbiology
University of Guelph, Guelph, Ontario, Canada
Ph.D. Microbiology 1990. University of Guelph, Guelph, Ontario, Canada
Thesis title: Development of vaccination strategies for brook trout against
infectious pancreatic necrosis virus (IPNV).

Professional Experience:

1994-present Research Scientist, DiagXotics, Inc., Wilton, CT
 Assistant Professor (courtesy), Dept. Microbiology, Oregon State
 University, Corvallis, OR
1992-1993 Research Scientist, MariGenetics, Inc., Corvallis, OR
 (part time)
1990-1994 Postdoctoral Research Associate, Dept. Microbiology, Oregon State
 University, Corvallis, OR
1985-1990 Laboratory Technician, Ontario Ministry of Natural Resources Fish
 (part-time) Health Laboratory, Guelph, Ontario, Canada

Publications:

- Bootland, L.M. & J.C. Leong. 1998. Infectious haematopoietic necrosis virus. In: Woo, P.T.K. & D. Bruno (eds.). Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections. CAB International, Oxon, UK. (in press)
- Leong, J.C., E. Anderson, L.M. Bootland, P.-W. Chiou, M. Johnson, C. Kim, D. Mourich and G. Trobridge. 1997. Fish vaccine antigens produced or delivered by recombinant DNA technologies. Dev. Biol. Stand. 90: 267-277.
- Leong, J.C., E. Anderson, L. Bootland, H. Carlson, P.-W. Chiou, M. Johnson, C. Kim and G. Trobridge. 1997. Cytokines and vaccines for aquaculture. In: New Approaches to Viral Diseases of Aquatic Animals, NRIA Intl. Workshop, Jan. 21-24, 1997, Kyoto, Japan.
- Bootland, L.M., P. Dobos and R.M.W. Stevenson. 1995. Efficacy of immunization of adult brook trout in preventing the IPNV carrier state and vertical transmission. J. Fish Dis. 18: 449-458.
- Bootland, L.M., N.M. Allen, K. Edwards, H.V. Lorz and L. Virk. 1993. Efficacy of immunization of rainbow trout fry with recombinant vaccines against IPNV. Bull. Aquacult. Assoc. Canada 93-4: 139-142.
- Leong, J.C., E. Anderson, L. Bootland, B. Drolet, L. Chen, C. Mason, D. Mourich, and G. Trobridge. 1993. Biotechnologic advances in fish disease research. Conference Proceedings of the International Marine Biotechnology Conference '91, Soc. Industrial Microbiology, Baltimore, Maryland, Oct. 13-15, Wm. Brown Communications, Iowa. pp. 573-586.
- Bootland, L.M. & J.C. Leong. 1992. Staphylococcal coagglutination - a rapid method of identifying infectious hematopoietic necrosis virus. Appl. Environ. Microbiol. 58:6-13.
- Bootland, L.M., P. Dobos & R.M.W. Stevenson. 1991. The IPNV carrier state and demonstration of vertical transmission in experimentally infected brook trout. Dis. aquat. Org. 10: 13-21.
- Bootland, L.M., P. Dobos & R.M.W. Stevenson. 1990. Fry age and size effects on immersion immunization of brook trout, *Salvelinus fontinalis* Mitchell, against infectious pancreatic necrosis virus. J. Fish Dis. 13: 113-125.

Curriculum Vitae of Co-PI: JO-ANN LEONG

TITLE: Emile Pernot Distinguished Professor & Chairperson of Microbiology
DEPARTMENT: Microbiology, Center for Salmon Disease Research
INSTITUTION: Oregon State University
ADDRESS: 220 Nash Hall, Dept. of Microbiology, Oregon State University, Corvallis, OR 97331-3804
TELEPHONE/FAX/EMAIL: (541)737-1834 or 1859/(541) 737-0496/leongj@orst.edu
EDUCATION: Ph. D., Univ. Calif. S.F. Med. School; Microbiology, 1971
B.A., Univ. Calif. Berkeley; Zoology, 1964.
POSITIONS HELD Chair, Dept. of Microbiology, Oregon State University, 1996-
Assist. Prof. to Distinguished Prof. Of Microbiology, Oregon State Univ., 1975-
President, Fish Health Section, American Fisheries Society 1996-97
PRESENT RESEARCH SUPPORT Sponsor
Development of Methods for Control of IHN in Commercially Reared Trout USDA, WRAC
Rational Immunotherapy -fish Sea Grant
Development of Caulobacters s Vaccine Presentation System Sea Grant-Marine
Biotech.
Effects of Endocrine Disrupting Chemicals on Immune System Sea Grant
Function in Estuarine Fishes (with C. Schreck, M. Fitzpatrick)
Expression Vectors for Genetic Immunization Sea Grant-Marine
Biotech.
A Delivery System for DNA Vaccines for Aquaculture (with M. Christensen) Sea Grant
SELECTED PUBLICATIONS
Anderson, E., D.V. Mourich, and J. C. Leong. 1996. Gene expression in rainbow trout (*O. mykiss*) following intramuscular injection of DNA. *Mol. Marine Biol. & Biotechnol.* 5(2):105-113.
Anderson, E., D.V. Mourich, S. Fahrenkrug, S. LaPatra, J. Shepherd, and J. C. Leong. 1996. Genetic immunization of rainbow trout (*O. mykiss*) against infectious hematopoietic necrosis virus. *Mol. Marine Biol. & Biotechnol.* 5(2):114-122.
Trobridge, G.D., P. P. Chiou, C. H. Kim, and J. C. Leong. 1997. Expression in vitro and in vivo of the Mx protein of rainbow trout by poly IC and IHNV. *Diseases of Aquatic Organisms* 30:91-98..
Trobridge, G.D., P.P. Chiou, and J. C. Leong. 1997. Cloning of the rainbow trout (*Oncorhynchus mykiss*) Mx2 and Mx3 cDNAs and characterization of trout Mx protein expression in salmon cells. *J. Virology* 71:5304-5311.
J.C. Leong, E. Anderson, L.M. Bootland, P.-W. Chiou, M. Johnson, C. Kim, D. Mourich, and G. Trobridge. 1997. Fish Vaccine Antigens Produced or Delivered by Recombinant DNA Technologies. R. Gudding, A. Lillehaug, P.J. Midtlyng, F. Brown (eds.). *Fish Vaccinology*. Dev. Biol. Stand. Basel, Karger 90: 267-277.
G. D.Trobridge, S. E. LaPatra, C. H. Kim, and J. C. Leong. 1997. Mx mRNA expression and RFLP analysis of rainbow trout (*Oncorhynchus mykiss*) genetic crosses selected for susceptibility or resistance to IHNV. *Diseases of Aquatic Organisms*, accepted. *Ore. Ag. Exper. Station Tech. Paper* No. 11,214.
Leong, J. C., E. Anderson, L.M. Bootland, P.W.Chiou, M. Johnson, C. Kim, D. Mourich, and G Trobridge. 1997. Cytokines and Vaccines for Aquaculture. In *Proceedings of Workshop on New approaches to Viral Diseases of Aquatic Animals*. ed. Y. Inui and J. Winton in Kyoto, Japan. January 21-24, 1997.

CURRICULUM VITAE of Co-principal Investigator: Mary R. Arkoosh

Education

Ph.D. in Microbiology - Oregon State University, Corvallis, 1989.

Graduate Training in Immunology Rush University, Chicago 1984.

B.S. in Biology, minor in Chemistry, Saint Mary's College, Notre Dame, 1983.

Professional Experience

1996-present Courtesy Faculty Member with the academic rank of assistant professor in the Department of Microbiology at Oregon State University, Corvallis, Or.

1995-present Research Advisor for National Research Council Postdoctoral Research Associate Program.

1989-present Group Leader of Immunology Team, Environmental Physiology Branch of the Environmental Conservation Division (ECD), NMFS.

Expertise

The Environmental Division of the NMFS is a leader in examining the effects of habitat alteration on fish physiology. We are committed to investigating the effects of contaminants on the immune response and disease resistance of juvenile chinook salmon. In a series of articles, we have demonstrated that juvenile chinook salmon exposed to contaminants are immunocompromised and have a lower resistance to disease. Our laboratory has extended these studies to Oregon where we demonstrated that pathogens are integral components of most coastal estuaries. Over all our studies suggests that disease is a natural occurrence contributing to the regulation of populations of salmon, and that anthropogenically induced factors may significantly shift the balance between salmon survival and mortality in the estuarine and ocean due to disease.

Relevant Publications

- ARKOOSH, M. R., E. CLEMONS, A.N. KAGLEY, R. OLSON, P. RENO, E. CASILLAS, and J.E. STEIN. 1998. Effect of pollution on fish diseases: Potential impacts on salmonid populations. *Journal of Aquatic Animal Health*. 10:182-190.
- ARKOOSH, M. R., E. CASILLAS, P. HUFFMAN, E. CLEMONS, J. EVERED, J.E. STEIN, and U. VARANASI. 1998. Increased susceptibility of juvenile chinook salmon (*Oncorhynchus tshawytscha*) from a contaminated estuary to the pathogen *Vibrio anguillarum*. *Transactions of the American Fisheries Society*. 127:360-374.
- E. CASILLAS, B. B. MCCAIN, M. ARKOOSH, J. STEIN AND U. VARANASI. 1997. Estuarine pollution and juvenile salmon health: Potential impact on survival. In Emmett, R. L., and M. H. Schiewe (editors). *Estuarine and ocean survival of Northeastern Pacific salmon: Proceedings of the workshop*. U.S. Dep. Commer., NOAA Tech. NMFS-NWFSC-29, 313 p.
- ARKOOSH, M. R., E. CLEMONS, M. MYERS and E. CASILLAS. 1994. Suppression of B-cell mediated immunity in juvenile chinook salmon (*Oncorhynchus tshawytscha*) after exposure to either a polycyclic aromatic hydrocarbon or to polychlorinated biphenyls. *Immunopharmacology and Immunotoxicology*. 16(2):293-314.
- ARKOOSH, M. R., CASILLAS, E., CLEMONS, E., MCCAIN, B. and U. VARANASI. 1991. Suppression of immunological memory in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from an urban estuary. *Fish and Shellfish Immunology*. 1:261-277.

CURRICULUM VITAE of Co-principal Investigator: Kym C. Jacobson

Kym C. Jacobson

National Marine Fisheries Service
Hatfield Marine Science Center
2030 S. Marine Science Dr.
Newport, Oregon 97365

EDUCATION:

Ph.D. Biology, Wake Forest University, North Carolina, 1991
M.S. Biology, Wake Forest University, North Carolina, 1987
B.S. Zoology, University of Nevada, 1985
B.A. French, University of Nevada, 1984

PROFESSIONAL EXPERIENCE:

1997-Present: Zoologist, NOAA/NMFS/NWFSC, Fish Ecology Division, Newport, Oregon
1996-1997: NRC Associateship Award Recipient, NOAA/NMFS/NWFSC, Environmental Conservation Division, Newport, Oregon
1993-1996: Postdoctoral Scientist, Seattle Biomedical Research Institute & Department of Pathobiology, University of Washington
1991-1993: Postdoctoral Research Fellow, Departments of Immunology and Neurology Mayo Clinic/Foundation, Rochester, Minnesota
1988-1991: Research Assistant, Department of Biology; Biology & French Tutor, Departments of Biology and Athletics, Wake Forest University
1985-1988: Teaching Assistant, Department of Biology, Wake Forest University

RESEARCH INTERESTS: Ecology of infectious diseases, community ecology, host-parasite interactions

SELECTED PUBLICATIONS:

- Esch, G.W., D.J. Marcogliese, T.M. Goater and **K.C. Jacobson**. 1989. Aspects of the evolution and ecology of helminth parasites in turtles: a review. In: The life history and ecology of the slider turtle, *Trachemys scripta* (J.W. Gibbons, ed.). Smithsonian Institution Press, p. 299-307.
- Jacobson, K.C.**, R.C. Fletcher and R.E. Kuhn. 1992. Resistance to complement-mediated lysis and differential binding of parasite-specific IgG to trypomastigotes of a Brazil strain of *Trypanosoma cruzi*. Parasite Immunol. 14, 1-12.
- Jacobson, K.C.**, R. Washburn and R.E. Kuhn. 1992. Binding of complement to trypomastigotes of a Brazil strain of *Trypanosoma cruzi*: evidence for heterogeneity within the strain. J. of Parasitol. 78(4), 697-705.
- Aho, J.M., M. Mulvey, **K.C. Jacobson** and G.W. Esch. 1992. Genetic differentiation among congeneric acanthocephalans in the yellow-bellied slider turtle. J. Parasitol 78(6), 974-981.
- Jacobson, K.C.**, J. de Oliveira Ferreira, M. de Fatima Ferreira da Cruz, J. Thurman, C. Schmidt, and R. Howard. 1998. A study of antibody and T cell recognition of *Plasmodium falciparum* rhoptry-associated protein 1 (RAP-1) and RAP-2 recombinant proteins and peptides in migrants and residents of the state of Rondonia, Brazil. Am J. of Trop Med Hyg 59:208-216.
- Howard, R. F, **K. C. Jacobson**, E. Rickel and J. Thurman. 1998. Analysis of inhibitory epitopes in the *Plasmodium falciparum* rhoptry protein RAP-1 including identification of a second inhibitory epitope. Infect Immun. 66(1):380-386

Section 10. Information/technology transfer

Information from this project will be distributed to the public, NMFS and the NWPPC in progress reports and by publishing research results in peer-reviewed journals. Researchers in this laboratory work closely with the ODFW pathologists and resulting data will be shared and discussed as it becomes available.

Congratulations!

—